

# Caribbean Cleaning Gobies Prefer Client Ectoparasites Over Mucus

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## Abstract

If cooperation often involves investment, then what specific conditions prevent selection from acting on cheaters that do not invest? The mutualism between the Indo-Pacific cleaner wrasse *Labroides dimidiatus* and its reef fish clients has been a model system to study conflicts of interest and their resolution. These cleaners prefer client mucus over ectoparasites – that is, they prefer to cheat – but punishment and partner switching by clients enforce cooperative behaviour by cleaners. By contrast, clients of Caribbean cleaning gobies (*Elacatinus* spp.) do not use punishment or partner switching. Here, we test the hypothesis that the behavioural differences between these two cleaner fish systems are caused by differences in cleaner foraging preferences. In foraging choice experiments, we offered broadstripe cleaning gobies *Elacatinus prochilos* client-derived parasitic isopods, client mucus and a control food item. The cleaning gobies significantly preferred ectoparasites over mucus or the control item, which contrasts with cleaner wrasses. We propose that the low level of cleaner–client conflict arising from cleaning goby foraging preferences explains the observed lack of strategic partner control behaviour in the clients of cleaning gobies.

## Introduction

The issue of conflict within cooperative and mutualistic partnerships has been a long-lasting interest among behavioural and evolutionary ecologists (e.g. Dugatkin 1997; Noë 2001; Sachs et al. 2004; Bshary & Bergmüller 2008). The marine cleaning mutualism involving the Indo-Pacific bluestreak cleaner wrasse *Labroides dimidiatus* and the numerous reef fishes (so-called clients) that regularly visit their territories (known as cleaning stations) to have their ectoparasites removed has proven to be a powerful model system for examining social conflict and partner control strategies (Bshary & Côté 2008). One of the main features of this model system is the existence of conflicts of interest owing to the foraging prefer-

ence of cleaner wrasses for client mucus (i.e. cheating) over ectoparasites (which constitutes cooperative behaviour) (Grutter & Bshary 2003). Therefore, cooperative behaviour by cleaner wrasses has to be promoted by clients. Several control mechanisms have been described, which include: the threat of reciprocation (e.g. death) by predators (Bshary & Bronstein 2004), partner switching or aggressive chasing (punishment) in response to cheating (Bshary & Grutter 2005) and prior observation to avoid biting cleaners (image scoring; Bshary & Grutter 2006). In return, cleaners try to manipulate client decisions by giving tactile stimulation with their pectoral and pelvic fins, typically to the clients' dorsal area. On the other hand, clients stay longer and are then less likely to leave following a conflict

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when they receive tactile stimulation from cleaners (Bshary & Würth 2001; Grutter 2004).

Recent research on other cleaner fish species strongly suggests that cleaner–client interactions are variable and that conclusions drawn from *L. dimidiatus* system should not be generalised (Soares et al. 2008a,b,c; Chapuis & Bshary 2010; Oates et al. 2010). One of the best studied alternative cleaner fish system is that involving Caribbean cleaning gobies. These cleaners share some of the characteristics of the *L. dimidiatus* system: cleaning gobies have small territories (i.e. cleaning stations) in which they receive fish clients that seek to have their parasites removed. Cleaning gobies interact repeatedly with many species of clients, and they prefer more parasitised clients over others (Soares et al. 2007). In addition, fish scales and mucus, which are indicative of dishonest cleaning, have been recorded in the stomach contents of cleaning gobies (Arnal & Côté 2000; Cheney & Côté 2005; Soares et al. 2008a, 2009). However, client control mechanisms such as punishment and partner switching, as well as any manipulatory or advertisement behaviour by cleaners, appear to be absent in the cleaning goby system (Soares et al. 2008c). Taken together, these studies suggest that conflict between cleaning gobies and their clients is reduced compared to the relationship between *L. dimidiatus* and its clients, but the causes of these differences remain unknown.

In this study, we tested the hypothesis that the differences between the cleaning goby and cleaner wrasse mutualisms could be attributed to differences in cleaner foraging preferences. *Labroides dimidiatus* is known to prefer client mucus over ectoparasites (Grutter & Bshary 2003), which creates conflict with clients. The lack of apparent conflict between cleaning gobies and their clients may therefore be attributed to an opposite foraging preference by cleaning gobies, i.e. ectoparasites over client mucus. If so, cleaning gobies would invariably begin an interaction cooperatively, and only cheat once their preferred food source is depleted. An initial cheating bite by the cleaner would then indicate to the client that it should leave the cleaning station, thus avoiding the need for other measures to ensure cleaner honesty.

## Methods

### Study Site and Species

This study was carried out in Barbados (West Indies) between August and September 2008. We focussed

on broadstripe cleaning gobies (*Elacatinus prochilos*), which is a small (up to 3.5 cm in total length), full-time cleaner that is easily recognised by the prominent white stripes that run laterally from the snout to the tip of the tail. These cleaners are abundant on Barbadian reefs. They may be found alone, in pairs or in groups, occupying cleaning stations on the surface of living coral (*Siderastrea* spp or *Montastrea* spp) or sponges. We only collected individuals living on coral as sponge-dwelling gobies clean less frequently (Arnal & Côté 2000; Whiteman & Côté 2002a,b). The cleaning gobies used for experimentation were all adults (seven males and six females), which ranged in total length from 2.3 to 3.1 cm.

### Food Preference Experiments

Client fish ectoparasites (i.e. gnathiid isopods and caligid copepods) and mucus were obtained from wild-caught coral reef fish, mostly parrotfish (*Scarus vetula*, *Scarus taeniopterus*, *Scarus iserti* and *Sparisoma aurofrenatum*) and surgeonfish (*Acanthurus bahianus* and *Acanthurus coeruleus*). Reef fish were caught in traditional Antillean fish traps and transferred to the laboratory where they were placed in aerated seawater-filled buckets for at least 1 h. Fluids were then filtered to collect detached ectoparasites. Mucus was obtained from four parrotfish of a single species (*Sc. taeniopterus*) that did not survive the collection protocol. These fish were immediately refrigerated, and the mucus was later scraped off with a scalpel blade (using the same method as in Grutter & Bshary 2003). All parasites and mucus were refrigerated after collection. Mucus in particular did not change in appearance and consistency after collection and refrigeration.

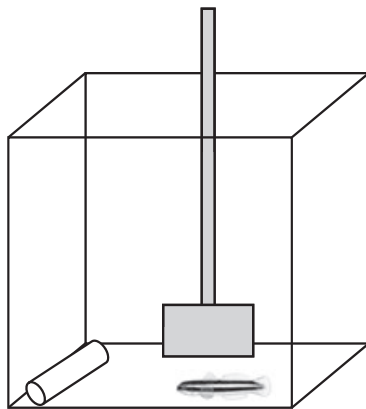
We tested the foraging preference (percentage of food items of each kind that were eaten) of 13 broadstripe cleaning gobies that were caught on the same reefs from which the reef fish yielding ectoparasites and mucus were obtained. Cleaning gobies were captured with hand-nets and maintained in individual aquarium compartments for 2–3 wk prior to the beginning of experiments. Captive cleaning gobies were fed mashed prawns and fish flakes spread onto white Plexiglas plaques (8 × 5 cm) to habituate them to the experimental feeding method. Each plaque had a grid of eight 4 × 2 cm cells painted on it. Glue, made of boiled flour and water, was used to fix the food items to the plaques.

Each cleaning goby was tested in its home compartment, twice on the same day, between 09:00 and 15:00, with at least 1 h between trials. In each

trial, we placed four ectoparasites randomly (caligid copepods and gnathiid isopods), two items of mucus and two items of boiled flour (control) on a grey gridded plaque, with each grid cell containing a single food item for a total of eight items per plaque. The position of each item was assigned at random to prevent positional biases or habituation. The surface areas of mucus or flour offered were similar (assessed visually using a binocular microscope). We also choose amongst the most size-suitable ectoparasites to be included in this study (as to make the surface areas of ectoparasites, mucus and flour as close in size as possible). Plates then were slowly placed inside each aquarium, against the wall opposite the experimenter, as carried out during the acclimation period (see Fig. 1). Cleaner foraging was recorded with a JVC Everio GZ-MG330 video camera that was placed 60 cm from the front wall of the aquarium.

### Statistical Analysis

We combined both types of ectoparasites into a single category. To control for the uneven frequency of encounter of the various food types (i.e. 50% parasites, 25% mucus and 25% control), we halved the observed number of parasites eaten. For each fish, we calculated the mean values for the two trials (trial 1 + trial 2). Following Grutter & Bshary (2003), we initially analysed the first two items eaten and then considered the first four items eaten. Using the first two items only allowed for the possibility of 100% preference for one food item over the others, while in the second analysis, with the four items, it is possible to obtain a higher reso-

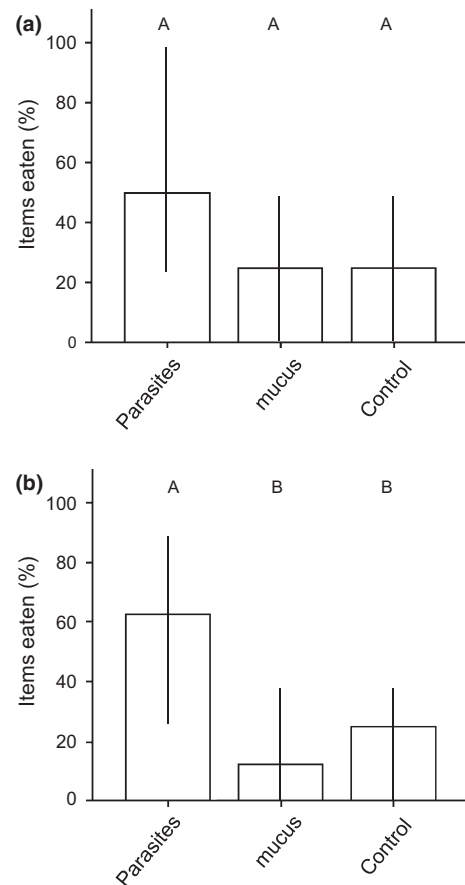


**Fig. 1:** Experimental set-up to test for gobies foraging preferences. Plates with all food items were slowly placed inside each aquarium, against the wall opposite the experimenter. Pipe served as shelter for fish.

lution for potential cleaner preferences between less preferred food items. The mean numbers of items of each type eaten across the two trials were analysed by using Friedman's ANOVA, followed by *post hoc* Wilcoxon matched-pairs signed-rank tests.

### Results

When only the first two items eaten were considered, cleaning gobies ate similar frequencies of all food types (Friedman's ANOVA:  $n = 13$ ,  $\chi^2 = 1.32$ ,  $df = 2$ ,  $p = 0.52$ ; Fig. 2a). However, when the first four items were considered, the frequency of consumption by cleaning gobies differed significantly among food types (Friedman's ANOVA:  $n = 13$ ,  $\chi^2 = 10.74$ ,  $df = 2$ ,  $p = 0.005$ ; Fig. 2b). *Post hoc* comparisons revealed that cleaning gobies consumed



**Fig. 2:** Percentage of all three food types eaten by cleaning gobies when (a) the first two items eaten are considered and (b) the first four items eaten are considered. Medians are shown, and error bars are interquartiles. Medians with different letters were significantly different from each other in Wilcoxon matched-pairs signed-rank tests ( $p < 0.05$ ).

ectoparasites more often than mucus or the control substance (Wilcoxon tests:  $p < 0.01$ ). Cleaning gobies ate mucus and the control substance at similar frequencies (Wilcoxon tests:  $p = 0.17$ ).

## Discussion

Our results demonstrate that cleaning gobies differ from cleaner wrasses in a key aspect concerning interactions with their clients: gobies have foraging preferences that will lead to a mutualistic outcome. The preference of cleaning gobies for ectoparasites reduces the potential for conflict with clients and may explain the large differences noted between the two cleaner fish systems in partner control and manipulation behaviours (Bshary & Côté 2008; Soares et al. 2008c).

Differences in foraging preferences between cleaning gobies and cleaner wrasses are unlikely to be explained by differences in the mucus quality as in both cases parrotfish mucus was used (Grutter & Bshary 2003; this study). Indeed, one could speculate that differences in foraging preferences could arise from interspecific variation in mucus quality, as fish mucus used in both studies was collected from different species of parrotfish. However, as shown by Arnal et al. (2001), mucus quality scored equally high quality indices amongst different species of parrotfish; thus, little variation in mucus quality is expected to exist across species of parrotfish.

The preference of cleaning gobies for ectoparasites became clear, when half of the items offered in each trial had been consumed. It is important to note that the cleaning gobies had no prior training, other than being habituated to feed from plaques, and hence could not know what to expect during the experiments. In fact, the absence of a significant foraging preference when only the first two items eaten per trial were considered suggests that gobies are capable to learn relatively quickly within trials, and as a result, they became increasingly focused on ectoparasites.

Interestingly, cleaning gobies do cheat under natural conditions. Stomach content analyses have shown that they not only feed on ectoparasites but also on mucus and scales, which constitutes cheating (Arnal & Côté 2000; Cheney & Côté 2005; Soares et al. 2008a, 2009). Moreover, clients jolt regularly during interactions with cleaning gobies, which reflects cheating by cleaners (Bshary & Grutter 2002; Soares et al. 2008b). However, the extent of cheating by cleaning gobies, as measured by the number of fish scales ingested, is negatively

correlated with ectoparasite availability (Cheney & Côté 2005). Taken together, these lines of evidence suggest that cleaning gobies may start interactions cooperatively, by searching for ectoparasites, but as this food source becomes depleted, they switch to ingesting mucus and scales (see Soares et al. 2008c for a similar argument). If so, then an infested client faces a low risk of being cheated at the onset of a cleaning interaction, but the first cleaner bite leading to a jolt is a reliable signal that its parasite load has been lowered and that it is time to leave the cleaning station.

In conclusion, our results provide the clearest evidence yet that all marine cleaning mutualisms are not identical. Differences may arise dependent on the presence or absence of a fundamental conflict between clients and cleaners over what the latter should eat. The sophisticated behaviours of the cleaner wrasse *L. dimidiatus* mutualism (Bshary 2006; Bshary & Côté 2008), which are absent in cleaning gobies, can only evolve within a context of a higher level of conflict between interacting partners.

## Acknowledgements

We thank the Director (Dr Judith Mendes) and staff of Bellairs Research Institute for logistical support. We also thank Stanton Thomas for support and friendship during the collection of fish ectoparasite samples. MSC was supported by a post-doctoral fellowship from Fundação para a Ciência e a Tecnologia (FCT) from the Portuguese Ministry of Science and Technology and SCC by a PhD fellowship from FCT. MS and RFO research is supported by a FCT grant (MAR-LVT-331/RG-LVT-331-2352). IMC is supported by the Natural Science and Engineering Research Council of Canada. RB is supported by the Swiss Science Foundation. All fieldwork expenses were financed by the Swiss Science Foundation. The data collection complied with the ethical laws of Barbados.

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